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14. ABSTRACT

The basis of this project is our finding that the Hsp90 co-chaperone, FKBP51, is upregulated at both the protein and mRNA levels in androgen independent human prostate cancer cells, LAPC-4 AI. Using biochemical approaches we found FKBP51 promotes assembly of a "superchaperone" complex containing ATP-bound Hsp90, p23, and FKBP51. Overexpression of FKBP51 leads to formation of maximal number of Apo-AR complexes, thereby increasing the number of AR molecules that bind hormone. FKBP51 enhances AR-dependent transcription of genes including PSA in response to both androgens and androgen-antagonists. Over-expression of FKBP51 enhances both AI and AD prostate cell growth in culture. Our working model is that FKBP51 determines androgen responsiveness in prostate cancer cells. By increasing the number of functional AR molecules that form in a low-androgen environment, over-expression of FKBP51 contributes to disease progression through androgen signaling and prostate cell proliferation. Drugs to FKBP51 may provide a complement or alternative to existing prostate cancer therapies.

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No subject terms provided.

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INTRODUCTION:

Ligand binding to nuclear receptors including the AR is regulated by molecular chaperones. The central player in this reaction is Hsp90, an ATP-utilizing chaperone that interacts transiently with LBDs to stabilize a conformation that is appropriate for ligand binding (Pratt and Toft, 1997b). Less well understood are the co-chaperones that belong to the immunophilin class of proteins, FK506-binding proteins FKBP51 and FKBP52, and cyclosporin A binding protein Cyp40. These co-chaperones have N-terminal domains with peptidyl-prolyl isomerase activity, and C-terminal domains that contain three tetratricopeptide repeats (TPR) that mediate binding to Hsp90 (Ratajczak et al., 2003). The functions of FKBP51 and FKBP52, which are 70% identical at the protein level, have been studied mostly in the context of the PR and glucocorticoid receptor (GR). FKBP51 negatively regulates GR and PR activity by reducing hormone binding affinity (Denny et al., 2000). In contrast, FKBP52 enhances GR, PR, and AR responsiveness to cognate hormone (Riggs et al., 2003). FKBP52 knockout mice have developmental defects in reproductive tissues (in males) consistent with reduced AR signaling and a failure of embryo implantation (in females) consistent with reduced PR signaling (Cheung-Flynn et al., 2005; Yong et al., 2007b). These observations together with the apparent absence of a phenotype in mice lacking FKBP51 led to the conclusion that FKBP51 does not play a significant role in AR signaling (Yong et al., 2007b). Here we show the Hsp90 co-chaperone FKBP51 is over-expressed in a xenograft model of AI prostate cancer, and describe a molecular mechanism for how FKBP51 promotes AR signaling in prostate cancer cells.

BODY:

PROGRESS WITH RESPECT TO THE STATEMENT OF WORK

Task 1 Optimize FKBP51 staining on paraffin-embedded samples (month 1).
Progress: FKBP51 antibodies have not, as yet, proven to be sufficiently specific and sensitive for TMA work, thus we are still trouble shooting these reagents with the hope of performing the TMA analysis. We have generated our own rabbit antibodies and affinity-purified them on recombinant FKBP51, and tested them by multiple assays to determine reactivity and specificity. This has been a major activity of this reporting period.

Task 2 FKBP51 immunocytochemistry on in-house TMA (months 1-3).
Progress: This task has not been initiated because it requires completion of TASK 1.

Task 3 FKBP51 immunocytochemistry on TMA from NCI (months 1-3).
Progress: This task has not been initiated because it requires completion of TASK 2.

Task 4 Statistical analysis of TMA results (month 4).
Progress: This task has not been initiated because it requires completion of TASK 3.

Task 5 Characterize growth rates of FKBP51 over-expressing lines in culture (low androgen, anti-androgens) (months 4-10).
Progress: COMPLETED IN PREVIOUS YEAR

Task 6 Measure sub-cutaneous tumor growth using FKBP51 lines (months 6-12).
Progress: Larger experiment (more mice) have been planned but not executed. We have generated cells lines stably depleted of FKBP51, which represent critical controls for the over-expression experiments. This has been a major activity of this reporting period.

Task 7 Intra-femoral injections with FKBP51 C4-2b lines, measure bone changes by Faxitron (months 10-16).
Progress: This experiment has not been performed because we want to complete the sub-cutaneous growth experiments before the bone experiments.

Task 8 Quantitative histomorphometry on bone experiments (months 16-21).
Progress: This TASK has not been performed because it depends on completion of the bone experiments.

Task 9 Test FKBP51 shRNA lines for sub-cutaneous and bone growth (months 18-24).
Progress: The cell lines needed for this experiment have been generated and partly characterized (see paragraph 4).

Task 10 Determine androgen sensitivity of FKBP51 lines, sub-cutaneous and in bone (months 18-21).
Progress: This work has not been initiated.

Task 11 Refine and repeat experiments, write manuscript, submit for publication (months 21-24).
Progress: COMPLETED IN PREVIOUS YEAR

Task 12 Use ligand binding assays to measure estrogen binding to AR (month 25).
Progress: This work has not been initiated.

Task 13 Use competition assays to determine Bmax and Ki values for anti-androgens in response to FKBP51 (months 25-27)**.**
Progress: Pilot experiments using a protein interaction assay described in Li et al 2010 were performed; a more rigorous approach with radiolabeled ligand is being planned.

Task 14 Use proteolytic digestion to measure Hsp90 conformational changes (months 26-27).
Progress: This work has not been initiated owing to difficulties with recombinant protein preparation.

Task 15 Determine if FKBP51 increases Hsp90 affinity for AR by biochemical reconstitution (months 26-28).
Progress: COMPLETED IN PREVIOUS YEAR

Task 16 Test whether FKBP51 regulates the ATPase cycle of Hsp90 by nucleotide exchange (month 27-29).
Progress: This work has not been initiated.

Task 17 Determine if FKBP51 inhibition sensitizes Hsp90 to 17-AAG for cell growth (months 29-33).
Progress: COMPLETED IN PREVIOUS YEAR

Task 18 Determine if FKBP51 inhibition sensitizes Hsp90 to 17-AAG for androgen binding and AR-dependent transcription (months 29-33).
Progress: This work has not been initiated.

Task 19 Refine and repeat experiments, write manuscript, submit for publication (months 33-36).
Progress: This work has not been initiated.

KEY RESEARCH ACCOMPLISHMENTS DURING THE REPORTING PERIOD:

1. Developed antibodies and several prostate cancer cell lines to analyze how FKBP51 promotes tumorigenesis.
2. Performed pilot experiments to study FKBP51 effects on tumor growth, and potential responsiveness to androgen depletion by castration.

REPORTABLE OUTCOMES

1. We developed two affinity-purified rabbit antibodies that show good specificity and sensitivity for FKBP51 detection by immunoblotting (Figure 1). Unfortunately these antibodies do not work on paraffin-embedded prostate specimens, which was the primary application intended for these reagents. Note a wide range of antigen retrieval methods were tested by our collaborator, a surgical pathologist with >20 years experience. It is fairly common for antibodies to work in multiple assays, but not by IHC on paraffin sections.

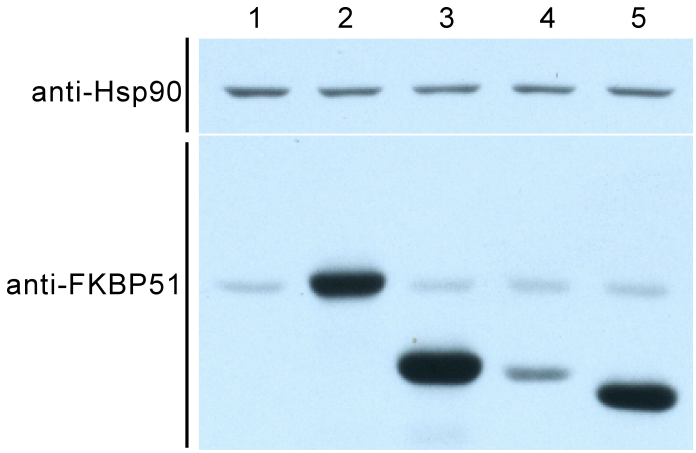

Figure 1. Characterization of affinity-purified FKBP51 antibodies. Full-length, recombinant hyman FKBP51 was injected into two rabbits over a course of four months, and the resulting anti-serum was purified on the same antigen immobilized on Sepharose beads. Affinity purified antibody (1 microgram/ml) was used to probe lysates from PC-3 cells transfected with the indicated forms of FKBP51. Lane 1, vector control. Lane 2, full-length FKBP51. Lane 3, FK1 domain deletion. Of FKBP51. Lane 4, FK2 domain deletion of FKBP51. Lane 5. TPR domain deletion of FKBP51. The antibody does not recognize FKBP51 in paraffin sections (data not shown). The second affinity-purified antibody gives identical results in the two assays.

2. We developed nine prostate cancer cell lines (including controls) with stably depleted/reduced levels of FKBP51 (Figure 2).

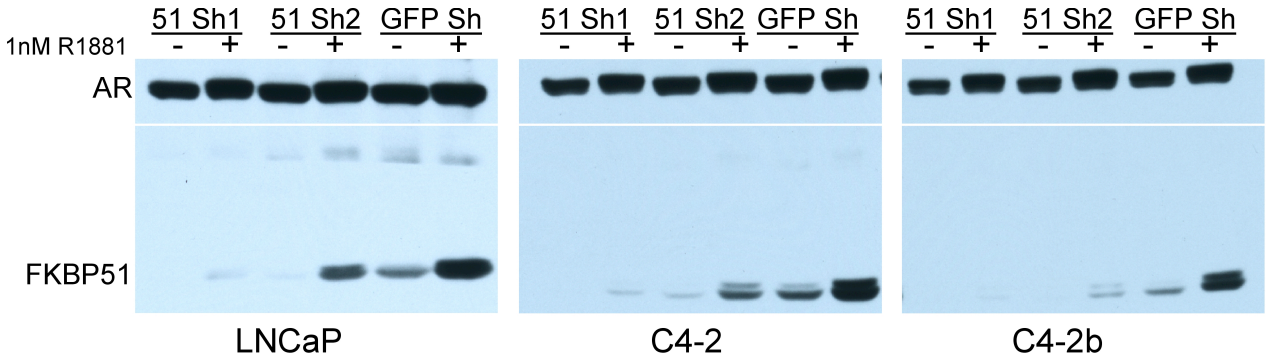

Figure 2. Development of prostate cancer cell lines with reduced levels of FKBP51. The LNCaP series was transduced with lentivirus encoding control (GFP) or FKBP51-specific shRNA (51 Sh1, 51 Sh2) and selected for antibiotic resistance (puromycin). Synthetic androgen treatment (R1881) for 24 hr induces FKBP51 expression, which is reduced to different extents by the two shRNAs.

3. We developed four LNCaP lines (including controls) stably over-expressing FKBP51 (Figure 3).

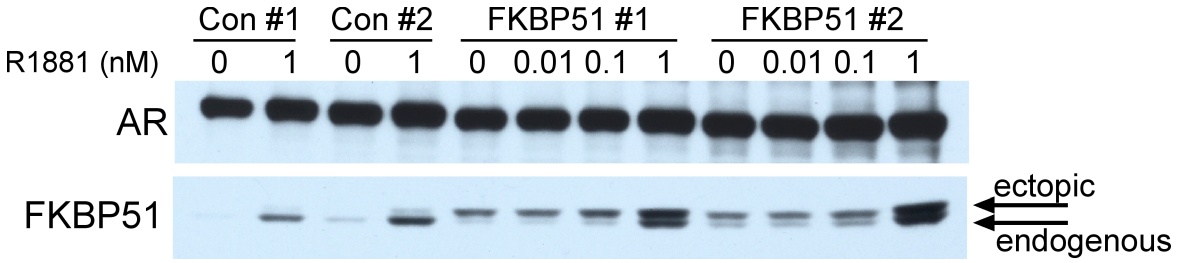

Figure 3. Stable over-expression of FKBP51 in LNCaP cells. Lentivirus encoding Flag-tagged FKBP51 was used to transduce celsl, and selection was performed in puromycin.

4. We performed pilot experiments to assess the affects of FKBP51 on tumor growth, including testing whether FKBP51 might help tumors survive in response to reduced androgen environment (Figure 4).

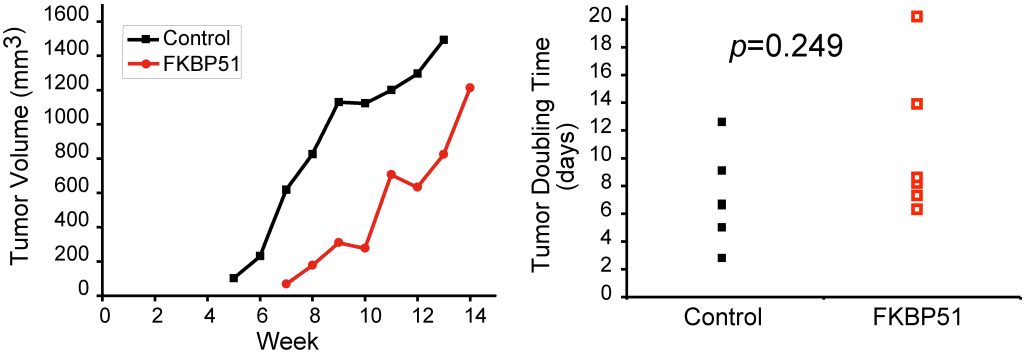

Figure 4. Growth of LNCaP-FKBP51 prostate cancer cells as xenografts in scid mice (6 mice/group). Control and FKBP51 over-expressing cells were injected sub-cutaneous (4 x 10^6) and tumor growth assessed by caliper measurement. Typical growth curves for control and FKBP51 cells are shown (left panel). There was variability in the latency of tumor establishment and growth that did not appear to be related to FKBP51 expression. The mice were castrated when the tumors reached ~600 mm^3; androgen depletion by this approach did not have an obvious effect in either set of mice. The doubling time for control and FKBP51-overexpressing tumors (right panel) appears to be increased slightly by FKBP51, but the effect is not statistically significant (unpaired t test). A larger experiment will be performed to rigorously address this question.

CONCLUSIONS

The antibodies we have developed are inadequate for IHC studies. To address this bottleneck we will determine if RT PCR methods can be used to interrogate FKBP51 expression on frozen prostate cancer samples (or possibly paraffin samples). The cell lines we have developed should be useful for analyzing FKBP51 contributions to cell growth, both in vitro and in vivo.

We have taken a highly focused approach that involved testing whether the expression of protein chaperones with known links to steroid hormone binding are altered in AI prostate cancer, and found that the co-chaperone FKBP51 is over-expressed in AI xenografts propagated in castrated mice. FKBP51 is a peptidyl prolyl isomerase that binds the immunosuppressants FK506 and rapamycin.

We found that FKBP51 promotes AR-dependent transcription using both reporter- and endogenous gene-based assays, consistent with published data (Febbo et al., 2005). Moreover, FKBP51 enhanced LNCaP cell growth in response to low (50 pM) androgen. The growth benefit of FKBP51 in LNCaP cells was inhibited by 17-AAG, which links FKBP51 to Hsp90 function. We obtained direct evidence that FKBP51 induces assembly of the Hsp90 superchaperone complex, and that this regulates the LBD of AR. This involves FKBP51 contact with Hsp90 and recruitment of p23, a co-chaperone known to stabilize Hsp90 binding to client proteins (McLaughlin et al., 2006). Crystallographic analysis revealed that p23 recognizes the ATP-bound, closed conformation of Hsp90; a major contact surface for p23 is formed by "lid" closure of Hsp90 (Ali et al., 2006). Thus, FKBP51 may promote ATP binding or lid closure, or it may stabilize another mobile element in Hsp90 structure such as the catalytic loop that is contacted by p23 (Ali et al., 2006). Hsp90 inhibitors are promising as therapeutics because Hsp90 regulates client proteins including AR that are critical for signal transduction and cell growth in cancer (Pearl et al., 2008). High dose 17-AAG, however, causes skeletal complications and increases Hsp70 levels, the latter of which may promote tumor resistance to therapy (Powers et al., 2008; Price et al., 2005). Hsp90 inhibitors used in combination with drugs that target co-chaperones that function in the same multi-subunit complex is a potential strategy for inhibiting clients such as AR that are highly dependent on the superchaperone complex. By promoting Hsp90 superchaperone complex assembly, FKBP51 has a quantitative effect on androgen signaling by increasing the number of AR molecules that are subsequently loaded with hormone. Because androgen-bound AR regulates FKBP51 expression, this creates a feed-forward mechanism that could amplify AR signaling under low hormone conditions that occur during androgen ablation. Our studies validate FKBP51 as a drug target in advanced prostate cancer.

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